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Original Investigation

Day-to-Day Variability in Spot Urine Albumin-Creatinine Ratio

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Background: Accurate quantification of albuminuria is important in the diagnosis and management of chronic kidney disease. The reference test, a timed urinary albumin excretion, is cumbersome and prone to collection errors. Spot urine albumin-creatinine ratio (ACR) is convenient and commonly used, but random day-to-day variability in ACR measurements has not been assessed.

Study Design: Prospective cohort study of day-to-day variability in spot urine ACR measurements.

Setting & Participants: Clinically stable outpatients (N = 157) attending a university hospital clinic in Australia between July 2007 and April 2010.

Outcomes: Spot urine ACR variability was assessed and repeatability limits were determined using fractional polynomials.

Measurements: ACRs were measured from spot urine samples collected at 9:00 AM on consecutive days and 24-hour urine albuminuria was measured concurrently.

Results: Paired ACRs were obtained from 157 patients (median age, 56 years; 60% men; median daily albumin excretion, 226 [range, 2.5-14,000] mg/d). Day-to-day variability was substantial and increased in absolute terms, but decreased in relative terms, with increasing baseline ACR. For patients with normoalbuminuria (ACR < 3 mg/mmol [<27 mg/g]), a change greater than ±467% (0-17 mg/mmol [0-150 mg/g]) is required to indicate a significant change in albuminuria status with 95% certainty; for those with microalbuminuria (ACR of 3-30 mg/mmol [27-265 mg/g]), a change of ±170% (0-27 mg/mmol [0-239 mg/g]) is required; for those with macroalbuminuria (ACR > 30 mg/mmol [>265 mg/g]), a change of ±83% (5-55 mg/mmol [44-486 mg/g]) is required; and for those with nephrotic-range proteinuria (ACR > 300 mg/mmol [>2,652 mg/g]), a change of ±48% (158-443 mg/mmol [1,397-3,916 mg/g]) is needed to represent a significant change.

Limitations: These study results need to be replicated in other ethnic groups.

Conclusions: Changes in chronic kidney disease status attributed to therapy or disease progression, when based solely on a change in ACR, may be incorrect unless the potential for day-to-day biological variation has been considered. Only relatively large changes are likely to indicate a change in disease status.
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INDEX WORDS: Albuminuria; albumin-creatinine ratio; proteinuria; chronic kidney disease.

Albuminuria (albumin excretion > 30 mg in a 24-hour period) is a marker of kidney disease in both diabetic and nondiabetic populations.[1] It also is a risk factor for cardiovascular events and cardiovascular and all-cause mortality.[2] Therapeutic strategies that decrease albumin excretion have been shown to delay the progression of kidney disease and lower the risk of cardiovascular mortality and morbidity.[3,4] Consequently, an accurate measurement of albumin excretion is crucial to stratify cardiorenal risk and monitor disease progression.

Semiquantitative tests to measure albuminuria, such as dipsticks, have suboptimal test specificity and sensitivity, which limit their clinical utility in the management of chronic kidney disease (CKD).[5] The reference test is albumin excretion measured from a 24-hour urine sample, which is cumbersome and subject to collection errors. A spot urine albumin-creatinine ratio (ACR) is a quick and convenient alternative and currently is advocated by key guideline groups.[1,6,7]

Spot urine ACR has been found to correlate well with 24-hour albumin excretion[8]; however, the extent of day-to-day variability in ACR at various magnitudes of albumin excretion is unclear.[9-11] When caring for patients with CKD, it is critical to know whether changes in ACR reflect biological variability in albumin excretion or a true change in disease status. We previously have reported that substantial day-to-day variability in spot urine protein-creatinine ratio (PCR) exists in individuals with CKD.[12] To quantify the

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day-to-day variability in spot urine ACR, we conducted a prospective study evaluating ACR measurements in paired samples obtained over 2 consecutive days in a cohort of patients with stable CKD.

METHODS

Study Design

We performed a study between July 2007 and April 2010 at a metropolitan tertiary-care teaching hospital in Sydney, Australia, which was designed and reported using the STARD (Standards for Reporting of Diagnostic Accuracy) guidelines.[13] The Sydney South West Area Health Service Ethics Review Committee approved this study, protocol number X06-0196.

Patient Recruitment and Consent

Patients were recruited from the hospital's CKD and kidney transplantation clinics. Eligible individuals identified from an electronic database were adults (aged ≥ 18 years) with albuminuria (ACR > 3.5 mg/mmol [>31 mg/g]) or proteinuria (24-hour urine total protein excretion >150 mg/d) with stable kidney function (outpatients with less than ±15% variation in estimated glomerular filtration rate [eGFR] over the preceding 3 months). Patients were excluded if they were on dialysis therapy, were known to be pregnant or less than 3 months postpartum, had symptomatic urinary tract infection, were treated for sepsis or hospitalized within the past 2 weeks, had overt cardiac failure, were menstruating, or were unable to provide informed consent. Participants provided written consent, and no financial incentives were provided.

Specimen Collection and Storage

Patients were given a urine collection kit containing 2 spot containers, a 24-hour urine collection (5-L) bottle, a sterile 10-mL plastic syringe, and written instructions for urine collection and storage. Participants were advised to continue their usual lifestyle, diet, and medications during the study period without changes, restrictions, or exclusions, in accordance with usual clinical practice.

Participants voided urine into a clean container at 9:00 AM and, using a syringe, transferred a 10-mL aliquot of this urine into a spot container and stored it at 1°C-4°C. On the following day at 9:00 AM, another spot urine collection was performed and stored using the same methods. The spot collections at 9:00 AM on both days were not first morning voids. All urine passed during the intervening 24 hours was collected in the 5-L sample bottle. Specimens were returned to the hospital the following day and analyzed in the hospital's centralized laboratory within 48 hours. No specimen was frozen. Participants underwent a blood test for hemoglobin, urea, and creatinine when urine specimens were returned. eGFR was derived using the isotope-dilution mass spectrometry–traceable 4-variable MDRD (Modification of Diet in Renal Disease) Study equation.[14]

The participant's blood pressure, height, weight, medications, and relevant medical history were recorded, and standard demographic information was collected from all participants. The data were de-identified before analysis and 10% of the entered data was randomly audited for accuracy of data entry.

Specimen Assay

The 24-hour specimens were assessed for adequacy. Any specimen with creatinine excretion <15 mg/kg/d in men and <12 mg/kg/d in women was regarded as incomplete and excluded from the study analysis.

The spot specimens were analyzed for albumin (milligrams per liter) and creatinine (millimoles per liter). ACR was derived by dividing the albumin concentration by the creatinine concentration, and the ratio was expressed as milligrams per millimole. Urine albumin was measured by a chemiluminescent enzyme immunoassay using an Immulite 2000 analyzer (Siemens). The analytical detection sensitivity limit for the urine albumin assay was 1 μg/mL. Laboratory within- and between-run coefficients of variation for urine albumin were 6% and 4.5%, respectively. Urine creatinine was measured by the kinetic Jaffé method on a Roche Hitachi modular analyzer. The detection sensitivity limit for urine creatinine was 360-57,500 mmol/L. For urine creatinine at concentrations of 5.39 mmol/L, laboratory within- and between-run coefficients of variation were 1.1% and 1.2%, respectively. Spot urine samples were not routinely cultured to detect bacteriuria because there is no convincing evidence that the presence of asymptomatic urinary tract infection significantly alters protein excretion rates.[15]

Statistical Analyses

The statistical significance of the mean difference between ACRs collected on consecutive days was determined using paired *t* tests, with 95% confidence intervals (CIs) and significance level at 0.05. Correlation between ACRs collected on consecutive days was measured using Spearman ρ.

We constructed Bland-Altman plots in which the difference of the measurements is plotted against the average of the measurements. We then calculated repeatability limits; that is, lower and upper limits in which 95% of the differences between 2 measurements on the same person should lie, using methods derived from those described by Bland and Altman.[16,17] First, we performed a regression using fractional polynomials of the absolute difference between measurements against the average of the methods. There was a small number of observations (n = 5) with an average ACR > 600 mg/mmol (all in the range of 600-1,500 mg/mmol [5,304-13,260 mg/g]). Because of the paucity of data in this range, we excluded these observations from the regression models. Thus, we restricted analysis to the 152 observations with ACR < 600 mg/mmol. Because of the possibility that the absolute difference between measurements may have depended on the level of measurement in a nonlinear manner, we used fractional polynomials in the regression.

We first fitted a fractional polynomial model with 2 powers, but because this was not significantly better than a model with a single power ($P = 0.06$), we used the model with single power. This model was $4\,|D| = 5.019 + 0.177 \times A$, where D denotes the difference of the 2 measurements and A denotes the average. The standard deviation (SD) of the differences is then given by multiplication by $\sqrt{(\pi/2)}$, which gives $SD = 6.290 + 0.222 \times A$; multiplying by 1.96 gives 95% repeatability limits of $\pm(12.328 + 0.434 \times A)$. This model provided reasonable fit, with 140 of 152 (92.1%; 95% CI, 87%-96%) of the observations lying within the repeatability limits (compared to an expected 95%, or 144 observations).

We tested whether the repeatability limits varied with age (stratified around a threshold of 55 years), sex, and eGFR category (<30, 30-<60, and ≥60 mL/min/1.73 m^2) by including a term for each of these variables in the regression equations.

The repeatability limits for test results were statistically extrapolated at different baseline ACR thresholds, if 2 or 3 repeat test results were available. Data were analyzed using Stata, version 12.1 (StataCorp LP).

We have previously published a similar analysis of day-to-day variability in spot urinary PCR.[12] We used those data to compare the day-to-day variability of ACR with PCR among 141 patients who were common to both analyses. We examined the correlation between ACR1:ACR2 and PCR1:PCR2 for each

patient and then compared their variability by plotting intrapatient test variability for ACR and PCR.

RESULTS

Patient Characteristics

Of the 570 patients from the hospital's CKD outpatient clinics who were invited to participate in this study, 270 consented and were enrolled. Forty-two percent (n = 113) were excluded because they did not provide all required specimens, leaving 157 patients whose paired samples were analyzed (Fig 1). The baseline characteristics of those who completed the study were not significantly different from those who did not. Patient characteristics are listed in Table 1. More than two-thirds of the study population was white. Median age of participants was 56 (range, 20-86) years and 60% were men. Median body mass index of participants was 27.7 (range, 17.8-51) kg/m^2, hypertension was present in 79%, diabetes was present in 32%, and 28% had a functioning kidney transplant. Median 24-hour albumin excretion was 226 (range, 2.5-14,000) mg/d, with 24 (15%) participants with normal-range (<30 mg/d) urinary albumin excretion, 58 (37%) with microalbuminuria (30-300 mg/d), and 75 (48%) with macroalbuminuria. During the study period, 122 (78%) participants were receiving either an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.

ACR Measurements

Mean day-1 ACR was 133.2 ± 223 (SD) mg/mmol (1,177 ± 1,971 mg/g) and mean day-2 ACR was 128.1 ± 199 mg/mmol (1,132 ± 1,759 mg/g). The difference in mean values between ACRs was not significantly different (5.1 mg/mmol; 95% CI, −2.9 to 13 mg/mmol [45 mg/g; 95% CI, −26 to 115 mg/g]; $P = 0.2$). As expected, correlation between ACRs collected on consecutive days was high (Spearman ρ = 0.95; Fig 2).

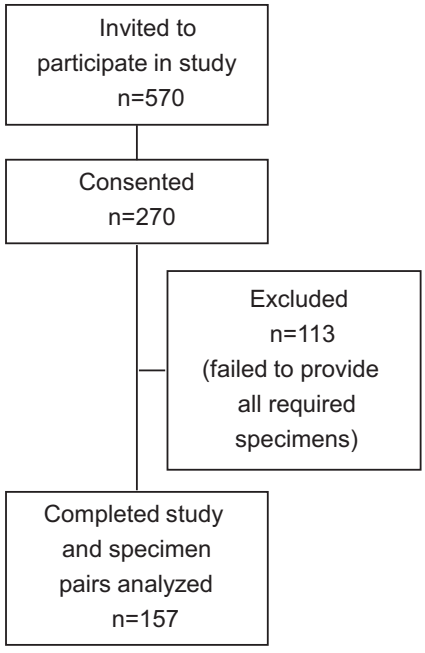

Figure 1. Study participants, flow diagram.

Table 1. Baseline Characteristics of Study Participants

Characteristic	Overall	24-h Albumin Excretion (mg/d) <30	30-300	>300
No. of participants	157 (100)	24 (15)	58 (37)	75 (48)
Age < 55 y	76 (48)	13 (54)	31 (53)	32 (43)
Male sex	94 (60)	10 (42)	36 (62)	48 (64)
Body mass index				
<18.5 kg/m^2	3 (2)	0 (0)	0 (0)	3 (4)
18.5-24.9 kg/m^2	43 (27)	8 (33)	15 (26)	20 (27)
25-29.9 kg/m^2	51 (32)	7 (29)	24 (41)	20 (27)
≥30 kg/m^2	60 (38)	9 (38)	19 (33)	32 (43)
Ethnicity				
White	124 (79)	20 (83)	42 (72)	62 (83)
Asian	19 (12)	3 (13)	8 (14)	8 (11)
Indian	7 (4)	1 (4)	5 (9)	1 (1)
Other	7 (4)	0 (0)	3 (5)	4 (5)
Systolic BP				
<120 mm Hg	33 (21)	6 (25)	17 (29)	10 (13)
120-<140 mm Hg	66 (42)	10 (42)	21 (36)	35 (47)
140-<160 mm Hg	46 (29)	6 (25)	18 (31)	22 (29)
≥160 mm Hg	12 (8)	2 (8)	2 (3)	8 (11)
Diastolic BP				
<80 mm Hg	62 (39)	10 (42)	27 (47)	25 (33)
80-<90 mm Hg	70 (45)	13 (54)	21 (36)	36 (48)
90-<100 mm Hg	17 (11)	0 (0)	7 (12)	10 (13)
≥100 mm Hg	8 (5)	1 (4)	3 (5)	4 (5)
Diabetes present	51 (32)	5 (21)	20 (34)	26 (35)
Cause of CKD				
GN	62 (39)	9 (38)	21 (36)	32 (43)
Diabetes	23 (15)	2 (8)	10 (17)	11 (15)
Other	54 (34)	13 (54)	20 (34)	22 (29)
Unknown	18 (11)	0 (0)	7 (12)	10 (13)
Kidney transplant	44 (28)	5 (21)	21 (36)	18 (24)
eGFR				
≥60 mL/min/1.73 m^2	51 (32)	8 (33)	30 (52)	13 (17)
30-<60 mL/min/1.73 m^2	66 (42)	12 (50)	20 (34)	34 (45)
15-<30 mL/min/1.73 m^2	37 (24)	3 (13)	8 (14)	26 (35)
<15 mL/min/1.73 m^2	3 (2)	1 (4)	0 (0)	2 (3)

Note: Values are given as number (percentage).

Abbreviations: BP, blood pressure; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; GN, glomerulonephritis.

Repeatability Limits of ACR

We generated repeatability limits of spot urine ACR (ie, the lower and upper limits in which 95% of repeat measurements on the same person in a clinically stable state should lie). The final model

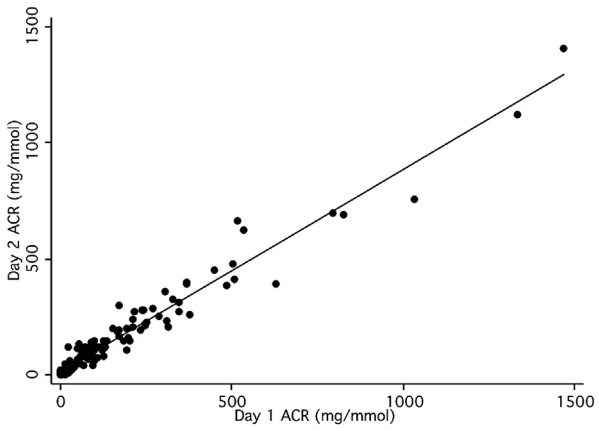

Figure 2. Scatter plot shows spot albumin-creatinine ratio (ACR) measurements from 157 patients with chronic kidney disease collected at 9:00 AM on 2 consecutive days. Spearman correlation $\rho = 0.95$.

generated was $\pm(12.328 + 0.434 \times A)$, where A is the average measurement (Fig 3).

The magnitude in the absolute difference of a repeat spot ACR result is determined by the magnitude of the baseline spot ACR, as shown in Fig 3. The absolute difference, or the repeatability coefficient between paired serial measurements, is expected to lie within ± 1.96 SD of the baseline measurement for 95% of paired measurements.[16] Therefore, in a clinically stable patient and at any baseline ACR, a repeat measurement can be expected to lie within the repeatability limits with 95% certainty. Thus, a repeat ACR test result that lies outside the 95% repeatability limits is likely to indicate a true change in disease status rather than just measurement error. In our study of 152 paired samples, the difference between pairs lay within the 95% repeatability limits for 140 pairs (92% of cases).

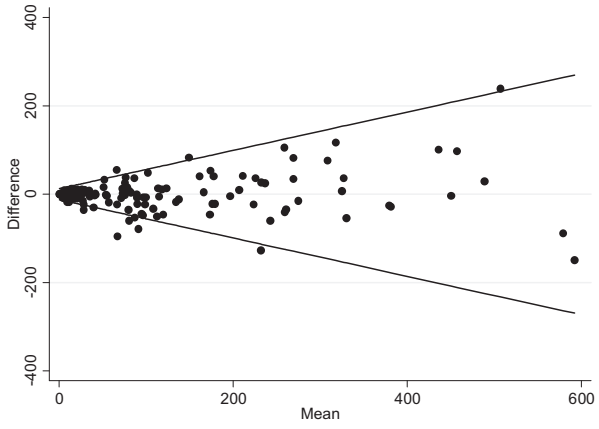

Figure 3. Variability in repeated spot albumin-creatinine ratio (ACR; mg/mmol) measurements from 157 patients with chronic kidney disease collected at 9:00 AM on 2 consecutive days. Fractional polynomial model shows the 95% repeatability limits of agreement between repeat spot urine ACR results. By regression, a fractional polynomial was generated to represent the 95% confidence interval within which repeat ACR measurements are expected to fall while the patient remains in a steady state. Note: this figure does not include participants with ACR > 600 mg/mmol (n = 5).

Table 2 demonstrates the wide range in expected variability of repeat test results at different ACR thresholds. In patients with microalbuminuria (ACR of 3-30 mg/mmol [27-265 mg/g]), the maximum range in variability for a repeat test result was comparatively large; for example, at a baseline ACR of 10 mg/mmol (88.4 mg/g), a repeat test result could range from 0-27 mg/mmol (0-239 mg/g), a change of $\pm 170\%$. However, in patients with macroalbuminuria (ACR > 30 mg/mmol [>265 mg/g]), for example, with a baseline ACR of 100 mg/mmol (884 mg/g), a repeat test is expected to fall within a range of 44-156 mg/mmol (389-1,379 mg/g), a change of $\pm 56\%$. Although the absolute range in variability was numerically greater for patients with higher baseline ACRs, when variability was assessed as a percentage change from baseline ACR, it progressively decreased in patients with high baseline ACRs such that for those with nephrotic-range proteinuria (ACR > 300 mg/mmol [>2,653 mg/g]), variability was less than $\pm 50\%$. The repeatability limits were not significantly different within the specified subgroups of age (<55 vs ≥55 years; $P = 0.8$), sex ($P = 0.2$), and eGFR ($P = 0.5$).

Statistical Extrapolation of ACR Test Reliability After Multiple Tests

The reliability of serial ACR results improved when more test results were averaged and compared with the baseline ACR (Table 2). Although the range in ACR repeatability limits decreased in proportion to the number of repeat tests, the effect was modest. For example, if baseline ACR was 30 mg/mmol (265 mg/g), the range in ACR repeatability limits if one repeat test was available was 5-55 mg/mmol (44-486 mg/g), the average of 2 repeat tests decreased the range in repeatability to 8-52 mg/mmol (71-460 mg/g), decreasing to 9-51 mg/mmol (80-451 mg/g) with 3 repeat tests.

Comparison of Day-to-Day Variability in ACR Versus PCR

We compared day-to-day variability in ACR and PCR among the 141 patients common to the present study and our previous publication on PCR variability.[12] The ACR and PCR day-1 to day-2 ratios for each patient, as measures of day-to-day variability in ACR and PCR, respectively, were highly correlated (Spearman $\rho = 0.78$; $P < 0.001$; Fig 4). In comparing variability as a percentage, in 70 (50%) cases, differences in variability were very similar for the 2 tests

Table 2. Day-to-Day Variability in Spot ACR by ACR Threshold

Baseline Range of Albuminuria	24-h Albumin Excretion	Baseline ACR	Repeatability Limits and Percentage Change From Baseline[a]		
			Single Repeat Test	Average of 2 Repeat Tests	Average of 3 Repeat Tests
Normal, <30 mg/d	~30 mg/d	3 mg/mmol	0-17 mg/mmol (±467%)	0-15 mg/mmol (±400%)	0-14 mg/mmol (±367%)
Microalbuminuria, 30-300 mg/d	~100 mg/d	10 mg/mmol	0-27 mg/mmol (±170%)	0-24 mg/mmol (±140%)	0-24 mg/mmol (±140%)
Macroalbuminuria, >300 mg/d	~300 mg/d	30 mg/mmol	5-55 mg/mmol (±83%)	8-52 mg/mmol (±73%)	9-51 mg/mmol (±70%)
	~1,000 mg/d	100 mg/mmol	44-156 mg/mmol (±56%)	52-148 mg/mmol (±48%)	55-146 mg/mmol (±46%)
Nephrotic range, >3,000 mg/d	~3,000 mg/d	300 mg/mmol	158-443 mg/mmol (48%)	177-423 mg/mmol (±41%)	184-416 mg/mmol (±38%)

Note: Conversion factor for ACR in mg/mmol to mg/g, ×8.84.

Abbreviation: ACR, albumin-creatinine ratio.

[a]Repeatability limits for ACR are the upper and lower values within which 95% of repeat ACR measurements in a clinically stable patient should lie. At all albumin excretion thresholds, variability decreases with multiple repeat measurements, improving the spot ACR test's reliability. Values in parentheses represent percentage change from baseline ACR corresponding to the repeatability limits.

Figure 4. Relationship between day-day variability in albumin-creatinine ratio (ACR) and protein-creatinine ratio (PCR). ACRs (day 1 to day 2) and PCRs (day 1 to day 2) for each patient, as measures of day-to-day variability in ACR and PCR, respectively, were highly correlated (Spearman $\rho = 0.78$; $P < 0.001$).

(within 10% of each other). In 42 (30%) cases, variability of ACR was >10% greater than PCR variability, though both were in the same direction. In 23 (16%) cases, variability of PCR was greater than ACR, though both were in the same direction. In only 6 (4%) cases were the variations in opposite directions, with a net difference >10%. No significant difference was evident in comparing variability in ACR with PCR (Wilcoxon signed rank test of ACR vs PCR day-2 to day-1 ratios [$P = 0.9$]).

DISCUSSION

Albuminuria is a well-established marker of kidney damage and is predictive of cardiovascular morbidity, mortality, and progressive loss of kidney function.[1,2,8] Thus, its measurement is important in the diagnosis and care of patients with kidney disease. Although several methods of measuring albumin excretion are available, each has its own limitations. Measurement of ACR in a spot urine sample is convenient for the patient, is less prone to collection errors compared with timed collections, and has been shown to correlate well with 24-hour urine albumin excretion.[8] Several clinical guidelines, including KDOQI (Kidney Disease Outcomes Quality Initiatives) and NICE (National Institute for Health and Clinical Excellence), recommend the routine use of spot ACR as a diagnostic tool to identify and monitor CKD.[1,6,7]

Interpretation of ACR results requires knowledge of test reproducibility over time. Our study demonstrates that spot urine ACR is subject to substantial day-to-day variability. Awareness of such variability in repeat ACR measurements may have important implications in the risk stratification and clinical management of patients with CKD and in clinical

research, when a spot ACR is used to quantify albumin excretion or when serial ACR results are used to monitor changes in disease status and response to therapy. At any magnitude of albuminuria, determining true changes in albumin excretion based on a single spot ACR measurement may be incorrect unless the change from baseline ACR exceeds the repeatability limit for that level of ACR. Failure to factor in such variability could lead to incorrect decisions in patient care or an incorrect interpretation of research.

Day-to-day variability in albumin excretion may occur due to endogenous or exogenous factors. Circadian variation in albuminuria has been well documented.[18,19] This may lead to changes in spot ACR measurements within any given day. Since we collected samples at 9:00 AM on both collection days, we eliminated this source of variation from our study. Changes in disease status and response to therapy can alter albumin excretion. We restricted this study to include only clinically stable patients whose medications were not being changed and we collected samples on consecutive days; hence, there was negligible risk of altered disease status in patients between sample collection time points. Because laboratory storage or measurement errors may contribute to variability,[20,21] we stored all samples at 1°C-4°C for no more than 48 hours and ran samples on a single analyzer, including paired samples in single runs in order to minimize any risk of this. The coefficient of variation of each laboratory measurement of urinary albumin and creatinine were small. Thus, we are confident that the day-to-day variation in spot ACR reported in this study represents inherent test variability under "ideal" circumstances. The test variability in spot ACR is likely to be of at least this magnitude in a typical clinical setting.

The range in ACR test variability differs substantially with the magnitude of albuminuria: although the absolute variability in ACR increases with the magnitude of albuminuria, as a percentage of baseline ACR, the variability decreases. For example, a patient with a baseline ACR of 10 mg/mmol (88.4 mg/g) will, on repeat testing, have an ACR of 0-27 mg/mmol (0-239 mg/g) with 95% certainty (range in variability, ±170%). For a patient with baseline ACR of 300 mg/mmol (2,652 mg/g), a repeat ACR will be 158-443 mg/mmol (1,397-3,916 mg/g) with 95% certainty (range in variability, ±48%). Therefore, a 100% change in ACR for a patient with microalbuminuria is likely to reflect test variability rather than a change in disease status, whereas a 100% decrease in ACR for a patient with macroalbuminuria is likely to indicate a true reduction in albuminuria. This is of major clinical importance because ACR thresholds may influence diagnostic and management decisions. We recently have published similar findings with regard to spot urinary PCR,[12] indicating that this degree of variability is not restricted to ACR. In comparing day-to-day variability between ACR and PCR among patients common to both studies, we found that variability between the tests typically is concordant and not dissimilar in magnitude, with no clear advantage in one test over the other in this regard.

To facilitate decision making in clinical practice, we have provided a series of reference ranges for repeat ACR measurements (Table 2). In stable patients, 95% of repeat measurements should fall within this range and measurements that fall outside this range may be indicative of change in disease status.

To determine whether ACR test reliability could be improved by repeat testing, we statistically extrapolated and compared the mean of 2 or 3 repeat test results to the baseline ACR (because it was not feasible in this study to conduct multiple repeat tests). We found that the range in variability narrowed; however, this improvement was modest at best and probably of little clinical utility (Table 2).

Our study has some limitations. Because we included only patients with stable kidney function in this study, this may induce selection bias and thereby limit the generalizability of our results. However, in doing so, we have effectively eliminated any changes in albumin excretion that could be attributed to changes in disease state, instead of simple test variability. The majority of participants in this study were white, and although our study population is representative of a typical CKD clinic, further prospective studies are needed to replicate our findings in other racial and ethnic groups. Because all participants in this study had CKD, our findings may not be generalizable to patients not known to have CKD when ACR is used as a diagnostic test. A minority ($n = 5$) of patients with very high-grade albuminuria (ACR > 600 mg/mmol [>5,304 mg/g]) did not fit within our regression models and were excluded; however, all within this group showed <20% day-to-day variability in ACR, which is consistent with our overall findings that variability expressed as a percentage of baseline ACR diminishes within increasing magnitude of ACR. A final note is that our study included only 2 consecutive measurements of ACR, and the ability of subsequent ACR measurements to reduce variability was estimated mathematically rather than measured.

Apart from its clinical utility, spot urine ACR is used widely in research settings. Although there was no significant difference between the mean values of repeat measures for the entire study cohort, our study results showed there was substantial variability at an individual level. Hence, spot ACR may be suitable for comparing the mean values between groups or at

different time points for a study population. However, caution is advised when spot ACR is used in studies with small sample sizes or when changes in the number of participants who exceed an ACR threshold are used as an outcome measure or to indicate a change in CKD stage.

Although spot urine ACR measurement is convenient, it is subject to substantial day-to-day variability. Such variability may limit the utility of spot ACR and should be kept in mind when it is used to monitor and care for patients with CKD in clinical practice and in research. We have provided tabulated repeatability limits at important thresholds of spot ACR to enable clinicians to determine whether changes in serial ACR results are likely to reflect test variability or a change in clinical status.

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